

Acoustic Experience Shapes Alternative Mating Tactics and Reproductive Investment in Male Field Crickets

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Summary

Developmental plasticity allows juvenile animals to assess environmental cues and adaptively shape behavioral and morphological traits to maximize fitness in their adult environment [1]. Sexual signals are particularly conspicuous cues, making them likely candidates for mediating such responses. Plasticity in male reproductive traits is a common phenomenon, but empirical evidence for signal-mediated plasticity in males is lacking. We tested whether experience of acoustic sexual signals during juvenile stages influences the development of three adult traits in the continuously breeding field cricket *Teleogryllus oceanicus*: male mating tactics, reproductive investment, and condition. All three traits were affected by juvenile acoustic experience. Males of this species produce a long-range calling song to attract receptive females, but they can also behave as satellites by parasitizing other males' calls [2]. Males reared in an environment mimicking a population with many calling males were less likely to exhibit satellite behavior, invested more in reproductive tissues, and attained higher condition than males reared in a silent environment. These results contrast with other studies [3] and demonstrate how the effects of juvenile social experience on adult male morphology, reproductive investment, and behavior may subsequently influence sexual selection and phenotypic evolution.

Results

Sexual signals can provide information about demographic parameters such as population density, the risk of intrasexual competition, and the average number of individuals nearby (mean crowding) [4]. Immature juveniles developing in an environment permeated by such signals may use them as cues that stimulate plastic changes in behavior or morphology optimized to the social environment likely to be encountered upon adulthood. To test this hypothesis, we manipulated juvenile experience of sexual signals in male field crickets (*Teleogryllus oceanicus*), some of which carried a silencing mutation, “flatwing.” We reared males either in silence or in the presence of male calling song. Juvenile acoustic experience strongly influenced the development of three traits in both flatwing and normal-wing adult males: the expression of alternative reproductive tactics, reproductive investment, and body condition.

Alternative Reproductive Tactics

Theory predicts that alternative male reproductive tactics (plastic changes in behavior or morphology) should be favored

when population density and mean male crowding are high and intrasexual competition is likely [5–12]. In *T. oceanicus*, males ordinarily call from stationary positions spread far apart (>1 m), but they can also behave as satellites by moving closer to other calling males [2]. Males reared in silence were more likely to exhibit such satellite behavior during playback trials than males reared with song; they spent approximately 77% more time in close proximity (within 50 cm) to a speaker broadcasting male song than males from an acoustically rich environment (Table 1; Figure 1). Older age intensified this effect (Table 1; Figure 1). The increased satellite behavior of males reared in silence contradicts the theoretical predictions mentioned above. However, it is consistent with prior work on *T. oceanicus* showing acoustic effects on plasticity in female mate choice, in which naive females were found to be more responsive to song playbacks than acoustically experienced females [13].

Males from both acoustic treatments were equally likely to approach the playback (binary logistic regression: $Z_7 = 0.13$, $p = 0.899$), indicating that they were equally able to perceive the signal and that any changes in behavior did not result from sensory inhibition as a result of exposure. Male movement toward the speaker during playback trials was consistent with satellite behavior and not aggression: we did not observe males stridulating, and those that responded were of poorer condition than those that did not (general linear model: $F_{1,395} = 6.00$, $p = 0.015$). The reverse would be expected if males responded aggressively, because aggression in male gryllids is associated with higher condition and the production of an aggressive song [14].

Male Reproductive Investment

Sperm competition models predict that males should increase their reproductive investment as sperm competition risk increases [15, 16]. Exposure to sexual signals that provide information about the abundance of sexually active males in the environment is therefore expected to mediate reproductive investment. *T. oceanicus* males that heard calling song during rearing invested nearly 10% more in reproductive tissue mass (testes and accessory glands) than males that experienced silence. Overall, larger males had greater reproductive tissue mass. However, reproductive tissues represented a significantly greater proportion of total body mass in males exposed to song even after statistically correcting for this scaling relationship (Table 1; Figure 2).

Condition

T. oceanicus males reared with song attained better adult condition than those that experienced silence, even though food availability and physical parameters of the environment were held constant (Table 1; Figure 3). Older age intensified this effect (Table 1; Figure 3).

Discussion

Juvenile social experience strongly influenced adult reproductive behavior and morphology in *T. oceanicus* males. Acoustic experience produced higher-condition males that invested

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Table 1. Results of General Linear Models Examining the Effects of Juvenile Acoustic Experience, Age, and Wing Morph on Satellite Behavior, Reproductive Investment, and Condition in Adult Male Crickets

	df	F	p Value
Satellite Behavior ($r^2 = 0.148$)			
Acoustic environment	1	20.42	<0.001
Age	1	7.08	0.008
Acoustic environment \times age	1	0.36	0.549
Condition	1	0.41	0.524
Error	183		
Reproductive Investment ($r^2 = 0.505$)			
Acoustic environment	1	17.34	<0.001
Age	1	2.14	0.144
Morph	1	7.72	0.006
Natural log (ln) soma mass	1	184.48	<0.001
Age \times ln soma mass ^a	1	3.75	0.071
Error	254		
Condition ($r^2 = 0.046$)			
Acoustic environment	1	18.38	<0.001
Age	1	2.51	0.114
Acoustic environment \times age	1	4.67	0.031
Error	484		

^a We left the age \times ln soma mass interaction term in the final model because the p value did not exceed 0.10 [38]; however, removing it does not qualitatively change the results.

more in reproductive tissues but were less likely to behave as satellites. These findings are consistent with previous work showing that poorer condition (or smaller) males tend to employ satellite or sneaking tactics [3, 17–22] but suggest that social experience transmitted via sexual signals in the environment is sufficient to alter condition, which we did not expect. Social experience alone may therefore independently influence condition, reproductive investment, and the adoption of alternative mating tactics. Alternatively, social

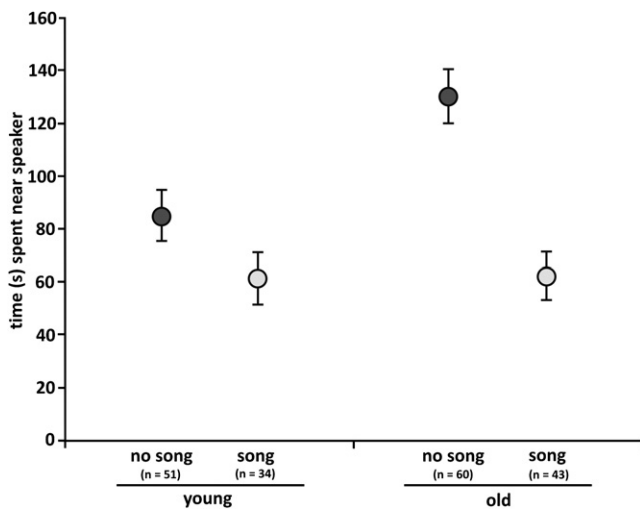


Figure 1. Acoustic Experience Decreases the Expression of Satellite Behavior

Satellite behavior in young (6 days posteclosion) and old (13 days posteclosion) males, measured as the amount of time (s) spent near a playback speaker during 5 min trials. Circles indicate means, and the bars indicate one standard error.

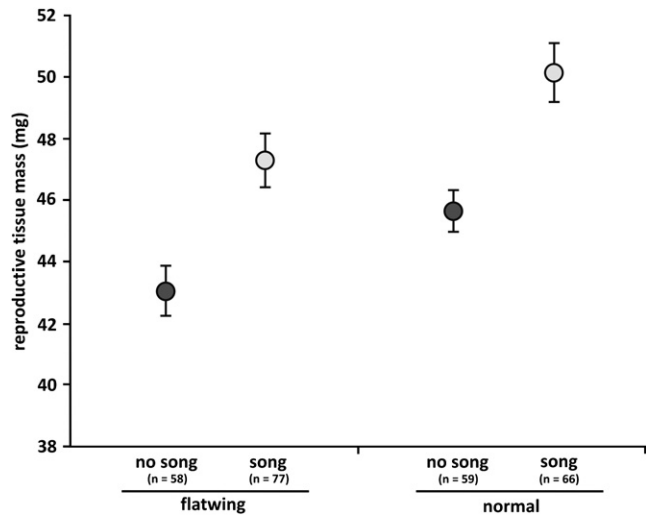


Figure 2. Acoustic Experience Increases Male Reproductive Investment

Reproductive investment is shown as the mass of reproductive tissues (testes and accessory gland). In our analysis, linear scaling between body mass and reproductive tissue mass was statistically controlled by using the analysis of covariance procedure of Tomkins and Simmons [37]. Circles indicate means, and the bars indicate one standard error.

experience might determine adult condition, which in turn influences variation in reproductive tactics and investment (see Figure S1 available online).

Social effects on reproductive behavior and morphology have the potential to impact sexual selection, but the impact depends on both the accuracy with which individuals gauge social cues in their environment and the extent to which they adjust plastic traits accordingly [1, 12, 23]. Our results illustrate both. In *T. oceanicus*, the cumulative effects of decreased satellite behavior and increased reproductive investment in populations with high mean male crowding, or at least the

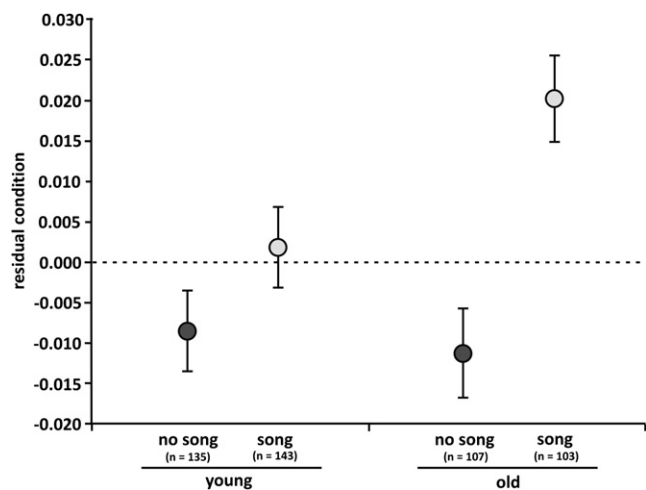


Figure 3. Acoustic Experience Increases Male Condition

Condition was estimated by using the residuals from a regression of body mass on pronotum length. The dashed horizontal line at $y = 0$ indicates average male condition. Circles indicate means for each group, and the bars indicate one standard error.

perception of high mean male crowding, would likely increase the strength of male-male competition and variation in mating success, thereby accelerating intrasexual selection. Our results also corroborate predictions made by quantitative genetic models of sexual selection that incorporate indirect genetic effects (IGEs): specifically, the prediction that IGEs can alter the tempo of sexual selection. For example, there is substantial additive genetic variation for male song in gryllids [24]. Genes influencing the production of song in calling males might thereby stimulate phenotypic changes in developing males who experience that song, further intensifying sexual selection by generating positive feedback between the social environment and the expression of male traits involved in competition for mates [25].

The response of male behavior and morphology to social experience is consistent with a predictive adaptive response, a term describing the process by which environmental cues from an animal's preadult environment optimize plastic traits to the environment it is likely to experience as an adult [26, 27]. Since 2003, over 90% of males on the Hawaiian island of Kauai, from which our animals were derived, have carried the silencing flatwing mutation [2]. The mutation appears to be maintained by pressure from a parasitoid fly, *Ormia ochracea*, and the Kauai population now experiences near silence as a result of the high proportion of mutant males [2]. Our results suggest that males developing in near silence on Kauai would be more likely to act as satellites to the remaining callers. Increased responsiveness to calling males would increase their encounter rate with females responding to those callers, and this behavior may have facilitated the establishment of the silencing flatwing mutation. Field studies support this hypothesis. Male crickets on Kauai have a greater tendency to move toward male song playbacks than crickets on other islands with fewer flatwings and a more signal-dense acoustic environment [2]. This facultative response to dynamic social conditions is likely to be beneficial regardless of whether or not a population contains flatwing males (social experience affected flatwing and normal-wing males equivalently in our experiment). The context in which males exhibit satellite behavior in this species—to increase encounter rate with potential mates—may explain why behavioral responses to acoustic experience, but not morphological responses, oppose theoretical predictions [5, 10].

Sexual signals in the environment of developing males appear to act as cues that stimulate plastic changes in reproductive behavior and morphology to match the social environment they are likely to experience as adults. In *T. oceanicus*, predictive adaptive responses in male reproductive traits may accelerate sexual selection through positive feedback from indirect genetic effects, and such plasticity may also have facilitated the establishment of the obligately silent flatwing morph [28]. Such responses may be adaptive in other contexts as well, including assessment of parasitism risk or competition for resources other than mates. The increased condition of males exposed to song, for example, could increase fitness if food resources become limited or migration becomes advantageous in a more crowded adult environment. However, animals experience a problem when the perception of their social environment does not match the actual social experience they are likely to have, and this system affords an excellent opportunity to test whether thresholds for socially mediated plasticity in the Kauai population of *T. oceanicus* remain fixed or evolve in response to the newly changed social conditions [29, 30].

Experimental Procedures

Rearing

Crickets were taken from an outbred laboratory population containing flatwing and normal-wing males. The population was established in 2003 with eggs from approximately 12 females from the Hawaiian island of Kauai and has been supplemented yearly with eggs from field-caught females. We reared individuals in an incubator at 25°C with a 12:12 hr light:dark cycle.

We isolated juvenile males at the stage preceding their penultimate instar, which is when sex differences become apparent. Males were kept individually in 118 ml plastic containers and were supplied with Purina rabbit chow and water ad libitum. Upon adult eclosion, which occurred approximately 2–3 wks after isolation, we surgically removed each male's elytral scraper to prevent him from singing and thereby confounding the experimental treatments. Flatwing males lack this structure but were treated with a sham operation as a control.

Treatment Groups

We randomly assigned isolated juveniles to one of eight treatments in a 2 × 2 × 2 factorial experiment, with acoustic environment (song or no song), wing morph (flatwing or normal), and age (young or old) as factors. Wing morphology can only be determined after adult eclosion. We estimated the proportion of flatwings expected to eclose based on their abundance in the adult population, which allowed us to isolate an appropriate number of male crickets in each treatment. Social effects might vary with age because of age-related changes in male reproductive behavior [31, 32]; alternatively, age-related variation might result from the longer exposure to different social environments that older crickets experienced. We therefore tested both young (6 days posteclosion) and old (13 days posteclosion) adult males. *T. oceanicus* males are capable of producing calling song and spermatophores by 6 days of age (unpublished data).

In the song treatment, individual crickets were placed in a Precision 818 low-temperature incubator with foam-lined shelves. Six male calling song models were simultaneously broadcast from six speakers at 70–80 dB sound pressure level (SPL) at the position of the crickets throughout the dark portion of the photoperiod (see [13] for methodological details). Sound pressure level was measured with an AZ Sound Meter (model 8922). Crickets in the no song treatment were placed in an identical incubator that lacked song. We swapped all treatments equally between the two incubators throughout the experiment to eliminate the possibility of incubator effects. We started the acoustic experience phase with juvenile males as opposed to adults, because in a closely related genus (*Gryllus bimaculatus*), the auditory cells of penultimate instars resemble those of adults and receive and integrate acoustic signals [33]. *T. oceanicus* breeds continuously, so nymphs are normally exposed to calling song in the wild.

Playback Trials

Trials were conducted in a 24°C–26°C anechoic room under red light. We performed them in a circular chamber 2 m in diameter with a Sony SRS-M30 speaker embedded in the center at floor level and foam lining on the walls to reduce echoing. Four inverted 118 ml plastic cups connected to a pulley system were spaced equidistantly around the perimeter of the chamber. The chamber floor was covered with crumbled leaf litter.

We placed a male under one of the four cups and allowed him to acclimate in silence for 2 min. We randomly varied males' starting points. An artificially constructed average male calling song was then broadcast from the central speaker at 70 dB SPL measured from 50 cm away, which approximates the intensity of a calling male [34]. We excised chirps from a song recorded in the wild and used them to construct a song with mean temporal and frequency parameters measured from a sample of wild-recorded songs. Details of *T. oceanicus* song structure, including an oscillogram, can be found in [35]. Once the playback began, we retracted the plastic cup via the pulley system and began timing. Each trial lasted 5 min.

We recorded whether or not the male responded to the playback by entering the circle 50 cm from the speaker and how long he spent inside the circle if he did. Our analysis focused primarily on the latter measurement. We chose 50 cm because that distance represents one standard error less than the mean distance at which satellite males were found near playbacks in the wild, thus providing a robust and biologically meaningful estimate of a male's tendency to exhibit satellite behavior [2]. We redistributed the leaf substrate between trials to disrupt odor cues deposited by males and washed the holding container and the surface immediately beneath it with 70% ethanol.

Morphological Measurements

After freeze-killing males, we obtained their total wet mass and reproductive tissue wet mass (testes plus accessory glands) to the nearest 0.001 g. We measured pronotum length to the nearest 0.01 mm.

Analysis

Statistical analyses were performed in Systat v.10. The likelihood of a cricket entering the inner circle near the playback was analyzed by using a binary logistic regression with a logit-link function with acoustic environment, wing morph, and age as factors, condition as a covariate, and the full complement of interaction terms. We then used a general linear model (GLM) to test how treatment, morph, and age affected male satellite behavior, which we defined as the amount of time spent within 50 cm of the calling song playback. The final GLM included acoustic environment, age, their interaction, and condition as a covariate.

We estimated relative condition by using the residuals from a regression of mass on pronotum length. We tested whether acoustic treatment, morph, and age influenced male condition by using a GLM. There is debate in the literature regarding the merits of mass-size residuals as a proxy for condition versus analyzing mass alone [36], so we analyzed both. The results were qualitatively equivalent, so we present just the analysis of residual condition. The final model included acoustic environment, age, and their interaction. To examine the possibility that high-condition males might have approached the speaker to engage in an aggressive encounter, rather than to engage in satellite behavior, we performed a separate GLM on condition with the same factors plus whether or not males responded during the trial. A separate GLM was run because our analysis of condition (above) included observations for which we did not also have behavioral data.

There is also debate in the literature about how best to estimate reproductive investment, because reproductive tissue mass is expected to scale with overall body size [37]. We adopted an analysis of covariance (ANCOVA) approach to analyze male reproductive investment above and beyond simple mass-scaling relationships [37]. We performed an ANCOVA on natural log-transformed reproductive tissue mass (testes plus accessory glands) with experience, morph, and age as factors and with natural log-transformed soma mass (whole body mass minus reproductive tissue mass) as a covariate. Including soma mass in the model corrected for body size differences between the treatments. ANCOVA analysis assumes that slopes of all groups are homogenous with respect to the covariate, which can be tested by examining covariate \times factor interaction terms [37]. However, to make accurate inferences about differences between treatment groups, nonsignificant covariate \times factor interactions must be removed from the final model [37, 38]. We removed interactions with a p value above 0.1, because covariate interactions with a p value below that may still indicate heterogeneous slopes [38]. The p value of the covariate \times soma mass term remained above 0.1; however, we ran the model with and without it, and the results did not differ qualitatively. The ANCOVA approach we use represents the most conservative of three techniques for estimating male reproductive investment reported in Tomkins and Simmons [38]; however, the other two, which we do not present here, yielded qualitatively equivalent results.

Supplemental Information

Supplemental Information includes one figure and can be found with this article online at [doi:10.1016/j.cub.2010.02.063](https://doi.org/10.1016/j.cub.2010.02.063).

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